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Systematic development and validation of stability indicating micellar electrokinetic chromatography methods for early stage drug candidates

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Abstract

Suitable methods for assessment of purity, potency and stability of new drug substances and drug products are required to be rapidly developed and validated to provide appropriate data for early project development decisions. In order to routinely provide methods of consistent and suitable quality to meet increasingly aggressive timelines, systematic approaches to both develop and validate analytical technologies have been developed. Systematic approaches to evaluate separation parameters such as buffer pH, buffer ionic strength, surfactant concentration, organic modifiers, organic modifier concentration, applied voltage and temperature were evaluated for an early stage drug candidate. Techniques to improve method precision and ruggedness were also examined. Finally, the validation results from the micellar electrokinetic chromatography method utilizing an internal standard were compared against the simultaneously developed high-performance liquid chromatography method.

Keywords: Validation; Stability studies; Pharmaceutical analysis

1. Introduction

Capillary electrophoresis (CE) and micellar electrokinetic chromatography (MEKC) methods have been shown to be useful technologies in the separation of pharmaceuticals and pharmaceutical related products and MEKC has been successfully utilized in separation of complex mixtures of both charged and neutral species [1–20]. Method precision, method ruggedness, limited dynamic range, and lack of experience in CE and MEKC as compared to HPLC have impeded the widespread use of these methodologies in the pharmaceutical analytical laboratory. In the past several years, a great deal of work has been done to address these issues particularly in the areas of method precision, sensitivity, and method ruggedness where data for CE and MEKC methods have been reported to approach those of HPLC [11,16,18,21–35]. Although it is highly unlikely that HPLC will be replaced by either CE or MEKC in the pharmaceutical analytical laboratory, these techniques provide a complementary tool for the analytical scientist to utilize to meet the ever increasing demands of pharmaceutical research and develop-

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ment. The combination of HPLC, CE, and MEKC allows for a rapid assessment of the purity, potency, and stability of new drug candidates to make higher quality, early development decisions.

The purpose of this work is to present a systematic approach for rapid development of MEKC methods for early stage drug candidates. MEKC method development and validation data for an early stage drug candidate stability-indicating method are discussed and the results are directly compared against the simultaneously developed HPLC method.

2. Systematic approaches to MEKC development

2.1. Initial feasibility assessment

General approaches to methods development have previously been reported [1,2,6,36]. During the years that CE and MEKC have been in use at Pharmacia, an easy to follow systematic approach to methods development has been developed. The systematic approach is designed to be utilized by an experienced laboratory staff that may not necessarily have expertise in CE or MEKC. Internal experts are available for assistance during the development process, if needed. Before initiating a method development effort utilizing CE or MEKC, an assessment needs to be made in regards to the feasibility/suitability of the technique to the separation. As in all separation methods, the intended use will drive the technology decisions and selection of technique. In Fig. 1, a set of basic questions in flowchart form are answered prior to any development activities. Samples that do not dissolve in aqueous buffer or aqueous surfactant solutions at concentrations of ~1–3 mg/ml are not suitable for CE or MEKC. Small amounts of organic, typically <30%, can be used in sample solvent and electrolyte [36–40]. In those cases where the percent of organic modifier approaches or exceeds 30%, another separation mechanism such as capillary

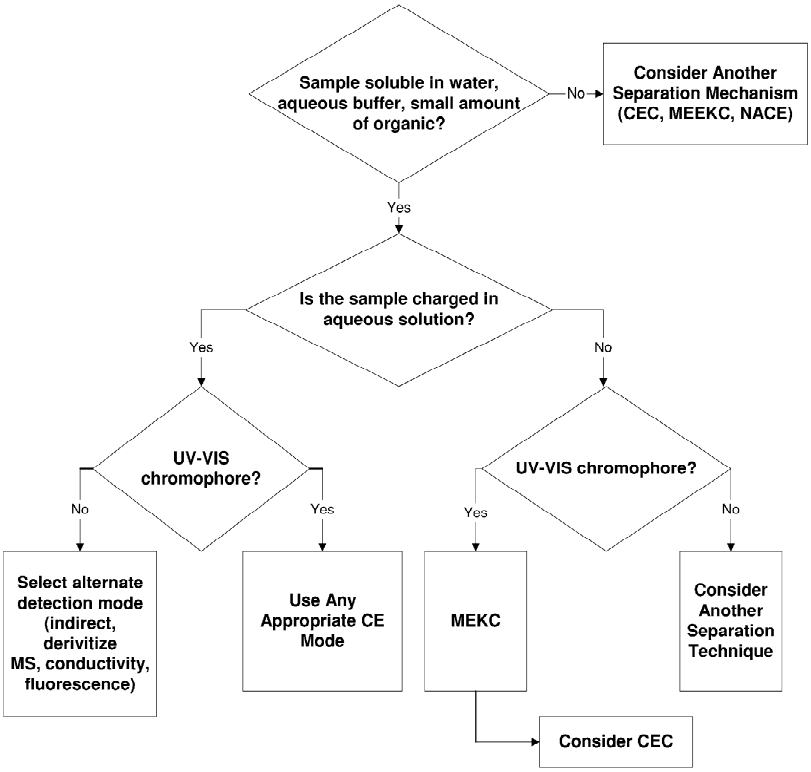

Fig. 1. Feasibility assessment flowchart.

electrochromatography (CEC), microemulsion electrokinetic chromatography (MEEKC), or nonaqueous capillary electrophoresis (NACE) may be a better choice [41–50]. The use of the initial assessment flowchart will reduce time spent in unsuccessful efforts to utilize CE or MEKC to meet a particular separation need in the early stages of drug development.

2.2. Preliminary experiments

If the initial assessment indicates that a mode or modes are feasible for further development, preliminary experiments are performed to obtain information pertaining to the separation. The preliminary experiments are designed to characterize the ionic nature of the sample components as well as to obtain an estimate of the mobility of the individual components. As indicated in Fig. 2, samples that contain both charged and neutral species will require MEKC as the appropriate mode for method development. Since very little is known about the ionic nature of the sample components in an early stage drug candidate, MEKC is often the mode of choice for initial methods development.

2.3. Optimize the MEKC separation

The optimization of an MEKC method, as shown in Fig. 3, begins with the selection of appropriate samples. It is important to ensure that all required samples are collected prior to initiation of development as this will reduce the amount of rework required. For impurity profiling and stability-indicating methods, samples typically include known or potential impurities, representative drug substance

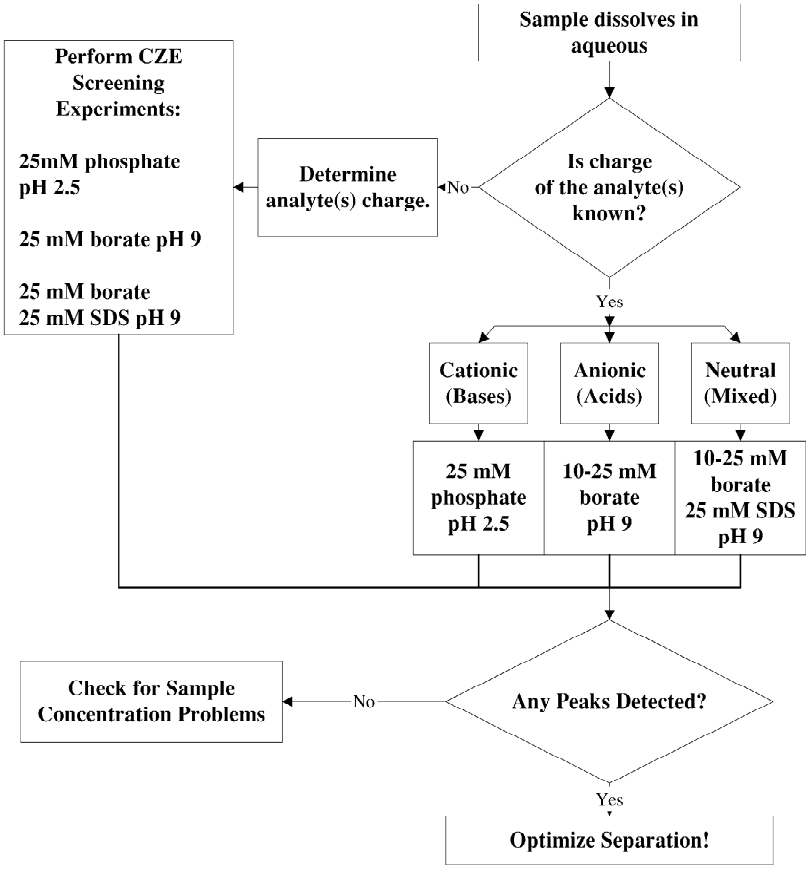

Fig. 2. Preliminary assessment flowchart.

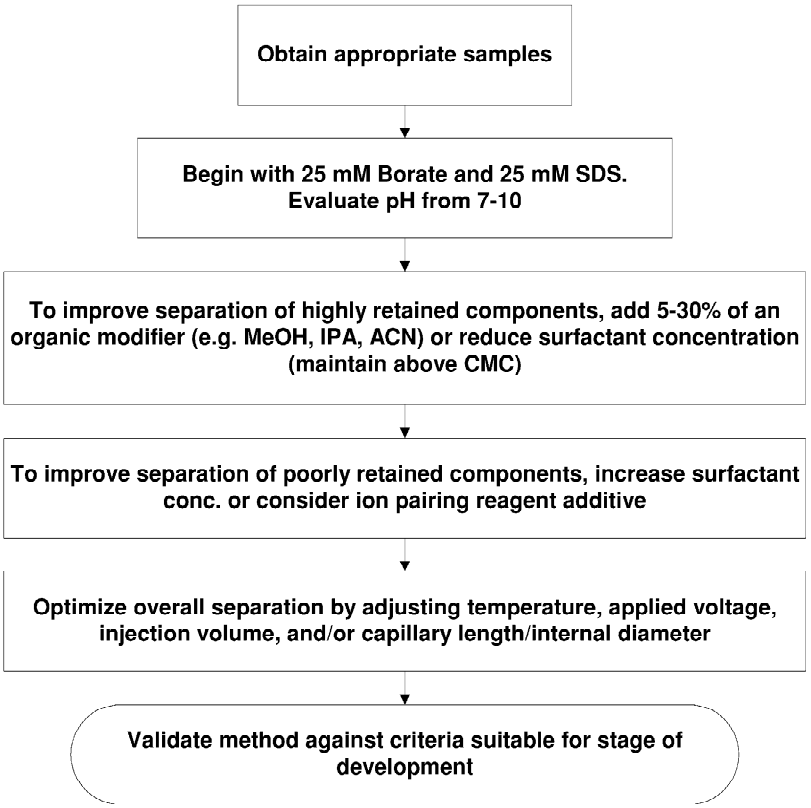

Fig. 3. MEKC rapid development flowchart.

lots, intermediate process isolates, and forced degradation samples. In early phase drug development, some of these samples may not be readily available. The recommended minimum samples required prior to initiating the development effort are representative lots and forced degradation samples.

One of the most powerful and important variables in achieving a separation in MEKC is the electrolyte pH [1]. For MEKC methods, the mobility of the analytes is determined across the pH range from 7 to 10. Typically, a plot of migration time versus pH will allow the selection of a narrow pH range for further investigation.

The role of surfactant is another critical parameter for evaluation [1,2,51–54]. Sodium dodecylsulfate (SDS), an anionic surfactant, is often selected for the initial evaluation with concentrations typically ranging from 50 to 150 m*M*. If the concentration exceeds 150 m*M*, problems with joule heating can be observed with the recommended ionic strength of 25 m*M* borate, which can negatively affect the quality of separation [1]. Also, cationic surfactants, such as cetyltrimethylammonium chloride (CTAC) and cetyltrimethylammonium bromide (CTAB) may also be examined to enhance selectivity [30,54].

If the drug candidate contained several highly retained components, the addition of small amounts of organic modifier such as methanol, acetonitrile or isopropyl alcohol may be added to improve/change selectivity [11,16,18,31,33,36–40,55]. It is recommended to begin by adding ~5–10% of the selected organic modifier to determine if any benefits are observed. The amount of organic modifier cannot typically exceed 30% in MEKC as compositions beyond this range will disrupt the formation of micelles in the electrolyte.

For some separations, it has been observed that the addition of an ion pairing agent, such as tetrabutyl ammonium phosphate, at concentrations of 5–25 m*M* has provided a significant improvement in the selectivity [1,16,56].

Other factors to consider to affect the separation

are use of different surfactants, such as bile salts, or complexing agents, such as cyclodextrin [1,2,57].

Although it appears as though many experiments are required to achieve a suitable separation, many of these experiments can be performed easily in the course of a few runs. The majority of the effort is in the preparation of the electrolyte solutions. If the instrument contains an autosampler, many variations in electrolyte composition can be evaluated within a single unattended run. Experimental designs can also be utilized to focus development efforts [33]. The typical development time utilizing the systematic approach, once all of the samples have been collected, is between 1 and 3 weeks. This is consistent with typical HPLC method development times for drug candidates in the early stages of development. It is recommended to either perform the MEKC and HPLC development in tandem or at a slight off-set in time, ~1 or 2 weeks. The benefit of the tandem or off-set approach is a more rapid and complete characterization of the early drug candidate. Many times the results of method development experiments in one technique will guide the decisions in development of the complementary method.

3. Experimental and methods

3.1. Chemicals and reagents

Acetonitrile, methanol, and isopropanol (HPLC grade) were obtained from Burdick and Jackson (Muskegon, MI, USA). SDS (CE grade) was obtained from Acros (Loughborough, UK). Sodium tetraborate and tetrabutylammonium phosphate (reagent grade) were obtained from Sigma–Aldrich (St. Louis, MO, USA). HPLC grade water was obtained from a Millipore Super-Q system (Bedford, MA, USA). Reagent grade hydrochloric acid (HCl) was obtained from EM Science (Gibbstown, NJ, USA) and reagent grade sodium hydroxide (NaOH) was obtained from J.T. Baker (Phillipsburg, NJ, USA). The drug substance candidate and corresponding parenteral formulation were obtained from Pharmacia Research and Development (Skokie, IL, USA). The drug substance candidate is an organic acid containing a mixture of charged and neutral impurities and degradation products.

3.2. MEKC instrumentation

The two CE instruments utilized in these studies were both Hewlett-Packard HP^{3D} CE systems (Palo Alto, CA, USA) equipped with online UV diode array detectors. Each system was controlled by Hewlett-Packard ChemStation software and a Hewlett-Packard personal computer. The data were collected on both Hewlett-Packard ChemStation and Perkin-Elmer TurboChrom chromatography data systems (Shelton, CT, USA).

3.2.1. Optimized MEKC conditions

The HP^{3D} CE instrument was programmed as follows: the detector was set at 215 nm, capillary temperature was 35 °C, the injection was hydrodynamic using 60 mbar for 6 s, the applied voltage was 30 kV in positive polarity. The typical observed current was ~45 μA. The running electrolyte and sample diluent was 5% isopropanol in 75 m*M* SDS–15 m*M* borate pH 9.0. The samples were prepared at 1 mg/ml in electrolyte solution. The run time was 30 min and the capillary was washed with 0.1 *M* NaOH for 3 min between each injection. The electrolyte vials were replenished or switched to another pair of unused electrolyte vials after every 4–6 injections.

3.2.2. MEKC capillary conditions

Untreated fused-silica capillaries 56 cm×50 μm I.D. equipped with bubble cells from Hewlett-Packard were utilized in all studies. The capillaries were conditioned prior to use by rinsing with 1 *M* NaOH, 0.1 *M* NaOH, and HPLC water. Capillaries were conditioned in electrolyte for 30 min to 1 h before use.

3.2.3. Buffer preparation

The buffers were prepared by dissolving the appropriate amount of SDS in buffer. Typically, 0.5 to 1 l was prepared and filtered for use in preparing sample diluent and running electrolyte. Since these relatively large volumes of buffer were prepared, significant losses of SDS on the filter were not expected. The buffer solutions were brought to the appropriate pH with sodium hydroxide or hydrochloric acid. The buffers were then filtered through a Millipore 0.45 μm HA filter and placed into instrument vials. In the electrolytes containing organic

modifiers, the organic modifiers were added to the buffers after filtration and subsequently placed into instrument vials. All electrolytes were vacuum degassed prior to use.

3.3. HPLC apparatus and optimized conditions

The HPLC system used was a Hewlett-Packard 1100 equipped with a diode array detector. The system was controlled by Hewlett-Packard ChemStation Software and a Hewlett-Packard personal computer. The chromatography data systems used were Hewlett-Packard ChemStation and Perkin-Elmer TurboChrom. The detection wavelength was set at 215 nm, the column temperature was set at 40 °C, and the flow-rate was 1 ml/min. A YMC Basic 250 mm×4.6 mm, 5 μm particle size was used and the mobile phase was acetonitrile–25 m*M* phosphate pH 2.5 (50:50, v/v). The sample concentration was 0.3 mg/ml in acetonitrile–water (50:50, v/v).

3.4. MEKC and HPLC sample preparation

For the drug substance, the drug powder was accurately weighed and transferred to an appropriate size volumetric flask. Appropriate sample diluent was added and flasks were shaken until the drug dissolved. The volumetric flasks were diluted to volume with the appropriate sample diluent and mixed well.

For the parenteral dosage form, samples were diluted utilizing appropriate pipettes, volumetric flasks, and sample diluents. Samples were mixed well after dilution to volume.

4. Results and discussion

4.1. MEKC separation

4.1.1. Effect of pH, ionic strength, and SDS concentration

The separation was affected by the electrolyte pH. The pH values examined were 8.0, 9.0, and 10.0. The separation at pH 9.0 was satisfactory for further optimization.

The ionic strength of the borate was examined at 10, 15, 25, 30, 50, and 100 m*M*. As ionic strength increased, retention times increased and peaks became noticeably broader. Evidence of joule heating was observed for the higher ionic strengths. The ionic strength did not significantly affect selectivity therefore the ionic strength of 25 m*M* was selected for further development to provide appropriate pH control and reduced joule heating.

The concentration of SDS was examined at 50, 75 and 100 m*M* using the previously selected 25 m*M* borate pH 9.0 buffer. The higher concentration of surfactant led to increased migration time and current. The concentration of 75 m*M* was selected for further optimization based on separation, run time, and current considerations.

4.1.2. Effect of organic modifier

Since the separation exhibited several later eluting components, the addition of 5–10% of the organic modifiers acetonitrile, methanol, and isopropanol was examined. Additions of 10% organic modifier increased run time, by reducing electroosmotic flow (EOF), and resulted in greater variability in migration times as compared to electrolytes with no organic modifier present. A degradation product of the parenteral dosage form was resolved from the parent only with the addition of 5% isopropanol.

4.1.3. Effect of applied voltage

The applied voltage was varied from 15 to 30 kV in positive polarity. The run times were effectively reduced by an increase in applied voltage. Evidence of joule heating, decreased resolution and peak shape, was observed at the higher applied voltages. In order to obtain the benefits of the shorter analysis time and to reduce joule heating, the ionic strength of borate was reduced from 25 to 15 m*M*. This change in ionic strength of borate addressed the joule heating issue without sacrificing selectivity or migration time reproducibility.

4.1.4. Effect of ion pair agent

The addition of ion pair agent was also examined. Approximately 20 m*M* of tetrabutylammonium phosphate was added to the 15 m*M* borate pH 9.0–75 m*M* SDS electrolyte. Some improvement in later eluting components was observed but similar im-

provements were observed with the addition of the organic modifiers.

4.1.5. Effect of temperature

The temperature of the capillary was examined at 30, 35, and 40 °C. The separation was affected by poor peak shapes and loss of resolution at 40 °C. The temperature of 35 °C was selected since it demonstrated suitable separation and run time.

4.2. MEKC electropherogram

Fig. 4 shows the qualitative comparison of optimized HPLC and MEKC methods. The sample was a mixture of force degraded samples, reaction intermediates, known impurities and representative lots. Some differences in selectivity between the two separation techniques can be observed. Overall, the profiles compare favorably indicating that both methods are stability-indicating for the drug substance and drug product samples. It is typically advantageous to have a single method, with suitable run time, that is applicable to both drug substance and drug product early in development.

In addition, since similar profiles are achieved with two complementary separation modes, specificity for each method is firmly established allowing greater confidence in each method's capabilities to provide the required high quality data.

4.3. MEKC method validation

MEKC methods have been validated consistent with ICH recommendations [11,16,18,31,33,58–61].

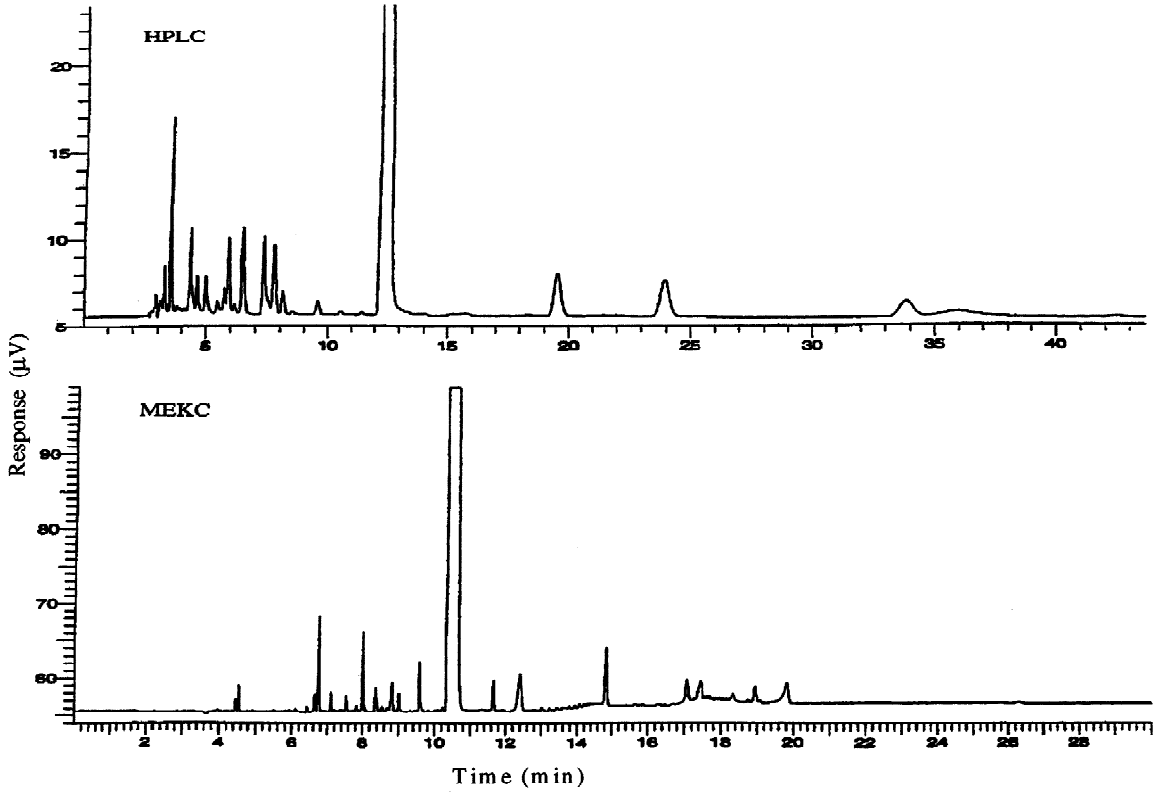

Fig. 4. Comparison of HPLC and MEKC separations. HPLC conditions: column, YMC-Basic 250 mm×4.6 mm I.D., 5 μm d_p; mobile phase, acetonitrile–25 m*M* phosphate pH 2.5 (50:50); flow, 1 ml/min; detection, UV 215 nm; temperature, 40 °C; run time, 45 min. MEKC conditions: capillary, untreated fused silica 56 cm×50 μm I.D.; running electrolyte, 5% isopropanol, 75 m*M* SDS–15 m*M* borate pH 9.0; injection, hydrodynamic 60 mbar for 6 s; applied voltage, 30 kV positive polarity; detection, UV 215 nm; temperature, 35 °C; run time, 30 min.

The optimized MEKC method utilizing an internal standard was validated using a one analyst–two run–three determinations per run experimental design. The drug candidate was utilized as a surrogate for impurities and degradation products at the appropriate levels.

4.3.1. Accuracy/recovery studies

Recovery was established at 0.1%, 0.5%, 1%, 80%, 100%, and 120% of the assay target concentration for the MEKC and HPLC methods. Mean recovery values for the MEKC method at impurity/degradation product levels of 0.1%, 0.5% and 1% were determined to be 117.5%, 108.8% and 104.8%, respectively. Mean recovery values for the MEKC method at assay levels of 80%, 100%, and 120% were determined to be 99.9%, 100.1%, and 100.2%, respectively. These values correspond favorably with the HPLC. The one minor exception is for the 0.1% level where the HPLC exhibited recovery of 103.6%. Overall, the performance of the MEKC was suitable for intended use.

4.3.2. Precision studies

Repeatability and intermediate precision, as RSD, were established at the same levels as reported in accuracy. Intermediate precision values for the MEKC method at impurities/degradation products levels 0.1%, 0.5% and 1% were determined to be 15.9%, 3.1% and 3.1%, respectively. For assay levels 80%, 100%, and 120% the intermediate precision values were determined to be 0.3%, 0.2%, and 0.4%, respectively.

These again compare favorably with HPLC with the minor exception of the 0.1% level where HPLC exhibited an intermediate precision value of 1.3%. The reduced precision in the MEKC method is attributed to signal to noise. In an effort to perform assay and impurities within a single injection of sample, peak areas of impurity levels are sacrificed in order to obtain improved accuracy and precision in the assay range. This reduced precision is not an issue for early development as the method still meets the intended use requirement.

In order to achieve these precision levels, an internal standard was found to be required. Use of an internal standard is commonly reported in quantitative MEKC methods [1,18,29–31,33]. The internal standard used was a propanamide salt compound being developed at Pharmacia with a purity of greater than 99%. The internal standard compound was previously found to be stable at the analysis pH. The impurities and major degradation product of the internal standard do not interfere with the analysis. Although stability of the internal standard was not examined in detail during method development or validation, no significant decreases in internal standard area or increases in the major degradation product were observed during the course of these experiments. Stability of the internal standard will be assessed in more detail later in development.

4.3.3. Linearity/range

The linearity of the MEKC method was established from 0.05% to 120% of the target concentration using levels of 0.05%, 0.1%, 0.5%, 1%, 80%, 100%, and 120%. Using Pharmacia developed linear regression software, full fit analysis calculated the slope, intercept, and correlation coefficient to be 1578 μV s ml μg^{-1}, 2164 μV s, and 0.99995, respectively. These values are consistent with the HPLC results.

4.3.4. Quantitation limit (LOQ)

The LOQ of the MEKC method was established at 0.1% of the assay target concentration. This is considered suitable for intended use in early phase drug development. In order to achieve a similar performance as HPLC, it is desired to eventually obtain LOQ values at the 0.05% level with this methodology. This represents a significant challenge to the routine application of MEKC to pharmaceutical analysis involving assay and impurities within a single injection of sample.

4.3.5. Detection limit (LOD)

The LOD based on three times signal to noise was determined to be 0.06% of the assay target concentration as compared to 0.01% by HPLC.

4.3.6. Specificity

Specificity was established through method development, comparison against the HPLC method, and parent peak purity by diode array. All known impurities, reaction intermediates, process impurities and degradation products are separated from the

main analyte and from each other. Placebo and forced degraded placebo parenteral formulation samples did not exhibit any significant interferences.

4.3.7. Robustness

Robustness for this stage of the drug candidate development was established through method development. Separation parameters such as electrolyte pH, buffer ionic strength, SDS concentration, organic modifier type, organic modifier concentration, applied voltage, and temperature were assessed. At this stage of development, the key separation parameters of temperature and organic content have been identified as variables that may require further study. Sample stability for 7 days was established at ambient, bench top conditions.

4.3.8. System suitability

The system suitability was established by RSD of multiple injections of standard throughout the course of the analysis. The MEKC method typically exhibits RSD values of 0.5–1.5% as compared to less than 0.7% for HPLC. The use of internal standard in MEKC allows for method precision to approach or meet that of HPLC. The system precision is suitable for the intended use of the method.

4.3.9. Method precision and ruggedness

Several techniques utilized to improve method precision and ruggedness included use of internal standard [18,29–31,33], thermostating of capillary, preconditioning capillaries prior to use [40] and optimizing injection plug length [22,23]. The plug length was optimized by adjusting sample concentration and injection pressure/time to achieve an appropriate amount on the capillary and a suitable plug length. The sample concentration of 1 mg/ml routinely is suitable to achieve appropriate plug length in a 50 μm capillary for the drug candidates studied at Pharmacia to date. Hydrodynamic injection is also the injection mode of choice to provide optimum injection precision. Internal standards are routinely required to achieve acceptable method precision.

Method ruggedness has been improved by filtering and degassing electrolyte solutions [34,35], using electrolyte as sample diluent, filling electrolyte vials to equivalent levels [21,24], changing either electrolyte vials or replenishing electrolyte solution after every few injections of sample [11,27,32], rinsing of capillary ends between injections to reduce carryover [25,26], ensuring capillary ends have a straight cut [28] and rinsing capillary with 0.1 *M* NaOH between injections. These actions require minimal effort by the laboratory scientist and can significantly improve the ruggedness of the methodology.

4.4. Comparative studies

Samples of actual drug product accelerated stability samples and representative drug candidate lots were assayed with both MEKC and HPLC methods. For the accelerated stability studies, two time points (4 weeks and 9 weeks) and four conditions (5, 30, 55 °C, and light) were studied. The results, as shown in Table 1, indicate that the data from the two techniques were indistinguishable on a practical level even at the low impurity/degradation product levels

Table 1
HPLC and MEKC accelerated drug product stability data comparison

Time point	HPLC					MEKC				
	Active	IMP	DEG 1	DEG 2	DEG 3	Active	IMP	DEG 1	DEG 2	DEG 3
Initial	102.0	0.3	–	–	–	102.0	0.3	–	–	–
4-A	102.5	0.3	–	–	–	102.6	0.3	–	–	–
4-B	102.6	0.3	–	–	–	102.2	0.3	–	–	–
4-C	102.3	0.3	–	–	–	102.3	0.3	–	–	–
4-D	100.6	0.3	0.5	0.2	0.1	100.6	0.3	0.6	0.2	0.1
9-A	101.6	0.3	–	–	–	101.1	0.3	–	–	–
9-B	101.7	0.3	–	–	–	101.4	0.3	–	–	–
9-C	101.7	0.3	–	–	–	101.7	0.3	–	–	–

Timepoints are 0, 4, and 9 weeks. Conditions are A, 5 °C; B, 30 °C; C, 55 °C; D, light.

for drug product accelerated stability samples. The comparative studies demonstrated that in practical use, the MEKC and HPLC methods are performing equivalently.

5. Conclusion

The use of a systematic method development approach allows for the rapid development and validation of stability-indicating MEKC methods that are suitable for early phase drug candidates. The validation results for the MEKC methods indicate suitable performance down to the 0.1% levels desired for qualification of impurities and degradation products at the early stages of drug development. These MEKC methods can be rapidly developed at the same time as the HPLC method without significant additional efforts. This tandem approach serves as a specificity qualification of the HPLC method and provides a more complete understanding of the purity and stability of early phase drug candidates. A more complete understanding of the drug candidate allows for better decisions and a reduced opportunity for surprises later in development. Surprises such as stability issues, unknown impurities or degradation products can negatively impact the development of a product in terms of cost and time. It is highly preferable to understand these potential issues completely before entering full development.

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